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A DISSERTATION FOR THE DEGREE OF MASTER

Measurement of cortisol levels in dogs
during animal-assisted interventions for
the evaluation of animal welfare

동물매개증재활동에 참여 중인 개의 동물복지
평가를 위한 코티졸 측정

2018년 2월

서울대학교 대학원
수의학과 수의생명과학 전공
김 준

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Measurement of cortisol levels in dogs
during animal–assisted interventions for
the evaluation of animal welfare

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A Dissertation submitted to
the Graduate School of Seoul National
University in
partial fulfillment
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Abstract

Although quality standards and hazard management for human subjects in animal-assisted interventions (AAIs) have been examined for years, only limited number of studies have explored the welfare state of involving animals. To set animal welfare standards for AAIs involving animals, studies on the stress level of the animals in on-the-job activities are needed. This study examined cortisol levels of the four AAI dogs to compare the stress level in different kinds of activities in the SAAA for children by volunteers with pets. To compare the stress level of AAI dogs according to the characteristics of the participants, the four dogs which have activities with three groups of intellectual disabilities (autistic adult, adult with intellectual disability, and youth with intellectual disabilities) were examined in AKCAAT. Although no significant changes in stress were observed in the saliva cortisol as physiological indicator, the activities demanding stronger human-animal relationships were noted to increase their stress, especially in young and inexperienced dogs. The finding of this study can give the insights for monitoring stress levels in AAI animals and setting the AAI animal welfare guideline in Korea.

Key words: AAA, AAI, working dog, Animal welfare, Stress, Cortisol

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List of Abbreviation

AAA: Animal–Assisted Activity

AAI: Animal–Assisted Interventions

AAT: Animal–Assisted Therapy

AAS: Animal Attitude Scale

AKCAAT: Association of Korean Companion Animal Assisted
Therapy

LAPS: Lexington Attachment to the Pets Scale

SAAA: Seoul Animal Assisted Activity

List of Contents

Abstract	1
List of Abbreviation	2
List of Contents.....	3
List of Tables	4
List of Figures.....	5
1. Introduction.....	6
2. Materials and Methods	12
2.1. Handlers and AAI dogs	12
2.1.1. AAS, LAPS Questionnaire	13
2.2. AAI design	19
2.2.1. SAAA	19
2.2.2. AKCAAT.....	20
2.3. Salivary sample collection	24
2.4. Sample analysis	28
2.5. Statistical analysis.....	28
3. Results.....	29
3.1. SAAA	29
3.2. AKCAAT	30
4. Discussion	35
5. Conclusion and animal welfare implications	46
5.1. Acknowledgments	46
5.2. Conflict of interest.....	46
6. References	47
7. Appendix	56
국문초록.....	58

List of Tables

Table 1. Classification and characteristics of AAI	7
Table 2. Descriptive demographics of the population of animal-assisted intervention participant dogs	15
Table 3. AAS scores of the participated handlers	16
Table 4. LAPS scores of the participated handlers	17
Table 5. Activities in the SAAA	22
Table 6. Participants of the AKCAAT	23

List of Figure

Fig. 1 Seoul Animal Assisted Activity Sampling Schedule	25
Fig. 2 Association of Korean Companion Animal Assisted Therapy Sampling Schedule	26
Fig. 3. Association of Korean Companion Animal Assisted Therapy Sampling Schedule in baseline setting	27
Fig. 4. Salivary cortisol levels of dogs in the SAAA by session ...	32
Fig. 5. Salivary cortisol levels of dogs in session 3 of the SAAA .	33
Fig. 6. Salivary cortisol levels of dogs in the AKCAAT by participant	34

1. Introduction

Because dogs have exceptional social skills and readily adapt to diverse human environments (Miklósi and Topál 2013), they are universally involved in animal-assisted interventions (AAIs) and seen as a source of motivation in health interventions, exercise, and social interactions for humans (Wilson and Barker 2003; Glenk *et al.* 2014).

AAIs are activities improving the psychosocial and emotional status of humans using animals (Barker *et al.* 2003) based on people's beliefs about the positive impact of human-animal bonds with dogs (Wilson and Barker, 2003).

AAIs can be classified according to purpose; Animal Assisted Therapy (AAT) for treatment purposes, and Animal Assisted Activity (AAA) focused on improvement of quality of life (Kruger and Serpell 2010), and Animal Assisted Education (AAE) for learning ability enhancement. Among them, AAA is participated by volunteers without specific treatment goals, unlike AAT which is conducted by medical professionals (Kruger and Serpell 2010).

Table 1. Classification and characteristics of AAI

	AAT	AAA	AAE
Objective	clear goals and treatment plans measurable form of improvement in physical and social cognitive functions (intentionally involving animals in treatment plans)	focus on improving the quality of everyday life	learning ability enhancement, Increased concentration of education
Facility	hospital, mental health treatment center, juvenile court, prison, nursing home	hospital, nursing home, child hospital	school, daycare center, special school
Target population	inmates, patients, elderly, children, child patients, AIDS patients	patients, vulnerable groups, elderly, children	students, children
Benefit	pain management, strengthening of social function, improvement of physical fitness, promotion of exercise activity, obesity suppression, increase of immunity, improvement of physical mental disorder	provide social support, reduce stress, increase activity	improve athletic ability, improve reading ability and communication dramatic behavior reduction
Examples	riding rehabilitation, horse riding therapy, prison abandoned dog training programs, pediatric hospital pain management programs	companion animal visiting programs	reading dog, educational support partners special education

The number of studies about the effects of AAIs on humans is growing, and various methods and their benefits have been investigated in the field of AAIs (Fine 2010). However, there are few studies on the effects on the human–animal bond and the welfare of animals during AAI (Hatch 2007).

The human–animal bond, which is a fundamental idea for AAIs, is a relationship that benefits both the health and well–being of humans and animals (AVMA 1998). Based on the concept of this relationship, animals in AAIs are partners and crucial components of the intervention process, not just the tools or rewards given to participants (Kruger and Serpell 2010). It would be undesirable to consider AAI–involving animals as merely risk factors to control. Therefore, the welfare of the animals is an essential condition for AAIs. It is important to expand the positive effects of human–animal bonds by identifying and improving negative elements of AAIs that can potentially threaten animal welfare. The welfare of dogs participated in AAA and AAT has been controversial because social interactions were claimed to be one of the most compelling stressors a dog could withstand (Von Holst 1998).

To assess an AAI animal' s welfare status, animal behaviour related to stress and the physiological indicators of stress as like cortisol are usually analysed (Vincent and Michell 1992; Beerda *et al.* 1999; Glenk *et al.* 2014). The level of cortisol can reflect the stress level of an animal. Although cortisol measurements do not demonstrate the

activity of the hypothalamic–pituitary–adrenal system itself, cortisol can be recognized as a critical indicator in measuring stress responses (Fries *et al.* 2009). Cortisol is secreted by the HPA axis and regulates body function to maintain homeostasis in unfamiliar situations (Fries *et al.* 2009). In general, it plays a role in adapting to adverse external environments, however, sustained external stimuli, if sustained in an increased amount of cortisol secretion, cause stress–related illness and adversely affect health (Chrousos 2009; Kooistra & Galac 2010).

Since salivary cortisol is proportional to the amount of cortisol in the blood (Kirschbaum 1989; Beerda *et al.* 1996), it is often used in non–invasive measurements. Because there is no restriction in observing changes in cortisol by salivary sampling alone, this method also can reduce stimulation during sampling compared with invasive sampling (Beerda *et al.* 1996).

There have been a number of studies on the measurement of cortisol during the AAIs. Haubenhofner (2006, 2007) and King (2011) found that cortisol levels were increased by an AAI in Austria and an AAT in hospital, but Glenk (2013, 2014) found that lead availability partially increased cortisol in an AAI in Austria. Zenithson (2014) did not observe cortisol changes during an AAA session functioning as a 60–min study break for college students in United States. There are studies in environments designed to identify factors that affect the experiment (Zenithson *et al.* 2014). In some studies, the content and

circumstances of AAI's activities have not been described, so there are limitations in understanding the impact of these variables (Haubenhofer 2007; King *et al.* 2011). The process of generalization in experiments is complicated because there is no standardized procedure or manual to cope with the change according to the target population, schedule, and activity target, compared with the diversity of interventions. Therefore, a detailed description of each AAI is warrant in studies evaluating welfare.

Identifying the welfare status of AAI animals should reflect the characteristics of and changes in human–animal relationships during the activity. For example, in AAIs targeting young children, it is possible that participants do not act gently or follow the instructions of the handlers, resulting in possible negative effects on the welfare of the animals (Schalamon *et al.* 2006; Marinelli *et al.* 2009). Therefore, a study to identify factors that could contribute to the welfare of dogs in on–the–job activities is needed.

In an AAI, the individual who can observe the animal's condition and take care of it is the dog handler (Serpell *et al.* 2010). Therefore, to investigate the impact of AAI participating handlers on the stress and welfare of participating dogs during an AAI is important. The handler's attachment to dog and attitude to animals can reflect the handler's impact on the stress and welfare of the AAI animals. They can be measured by examining the Animal Attitude Scale (AAS) and the Lexington Attachment to the Pets Scale (LAPS) (Johnson *et al.*

1992, Herzog *et al* 2015). AAS is widely used measures of general attitudes toward animal use and protection in humans (Herzog *et al.* 1991). AAS has been used to demonstrate attitudes, empathy toward animals and ethical orientation to animal use (Galvin and Herzog 1992, Taylor and Signal 2005). LAPS has the highest number of citations among the 140 tools used in human–animal interaction field (Wilson and Netting 2012). It is most widely used to Questionnaire to assess Human–animal bond, especially emotional attachment (Douglas 2005). Individual scores of LAPS were calculated according to each item and computed by General Attachment (LAPS–GA), Person Substitution (LAPS–PS), and Animal Rights (LAPS–AR) (Ramírez *et al.* 2014).

In Korea, AAI has been introduced and has spread in a short time without proper animal welfare guidelines for AAI animals, which can prevent any risks that AAI develops in a negative direction. In the United States and Australia, to become an AAI animal, a dog must undergo stringent selection and has to complete multiple levels of the special training and a temperament screening to meet the criteria established by authorized institutions (Davis 1992; Haubenhofer and Kirchengast 2006; Serpell *et al.* 2010). However, there is only a screening test that determines the qualification of the participating dog by simple behavioural evaluation of aggression and obedience without a training for specific situations for AAI in Korea. Therefore, assessing the working stress of the AAI animals is important to improve their welfare status.

The demographic characteristics such as the age of the participants and the way of handling type on dogs applied to the AAI affect the stress (Marinelli *et al.* 2009; Glenk *et al.* 2013). Marinelli argued children participant under 12 old can impact to AAI animal's stress expression and Glenk found lead wear variable make a significant effect on the change of cortisol in AAI dog.

Therefore, this study aimed at comparing the AAI dogs stress level in different kinds of activities and examining the stress level of AAI dogs according to the characteristics of the participants. In this study, two AAI programs were monitored for the influence of these factors on the saliva cortisol of AAI dogs. First is Seoul Animal Assisted Activity (SAAA)” Program, a public project for children of vulnerable classes by the Seoul Metropolitan Government. In the SAAA, I examined changes in cortisol according to the session content. Second is Korean Companion Animal Assisted Therapy (AKCAAT) AAT programs for people with Autism and the intellectual disabled. In AKCAAT, I monitored cortisol levels according to the characteristics of the subjects.

2. Materials and methods

All protocols of this study were approved by the Institutional Animal Care and Use Committee (IACUC; SNU-160831-1) and the Institutional Review Board (IRB; 1609/003-011) at the Seoul National University.

2.1. Handlers and AAI dogs

The subjects and AAI dogs were recruited during the AAI training program for the handlers by informing and emailing volunteer teams of SAAA and AKCAAT. To minimize the effect of the presence of the researcher during sampling, the dog became accustomed to this situation through a pre-sampling training and four AAI sessions.

To participate the SAAA program, handlers needed to complete 40 hours of an AAI training course. In AKCAAT, handlers were not volunteers. They should take AKCAAT Educational training course in 2 years for certification about “Companion Animal Assisted Psychology Counsellor” . Education training process includes 300 hours of training and 100 hours of AAI internship. Qualification requires a second grade social worker certificate and college graduation in the related field.

However, the involving animals only needed to pass a simple health inspection (no pain, no diseases, no parasites, and fully vaccinated) and an aggression and obedience test. Among the participant animals, I excluded dogs taking NSAIDs or medications that could affect cortisol levels (Tanaka *et al.* 1998; Gottschalk *et al.* 2011) within the last 6 weeks. I also excluded dogs who actively refused to participate in saliva collection. A total of eight participant dogs were selected as study subjects (Table 2, 3).

2.1.1. AAS, LAPS Questionnaire

For measuring handler’s attachment and attitude to animals, a 10-item version of AAS and 23-item version of LAPS were used in

survey for AAI handlers. Both version was translated into Korean using the back-translation method (Brislin 1970). The results of the two surveys were calculated through the sum of the items, and more top the score indicates the higher the attitude and attachment. Since items 2, 3, 4, 7 and 8 were reversed questions in the AAS, the inverted values were also used in the score calculation, and the response values for the items had the values from total disagreement (1) to total agreement (5). In LAPS, the response to the item ranged from total disagreement (0) to total agreement (3), with items 8 and 21 reverted. The scores of the LAPS were calculated in three parts: LAPS-GA (items 10, 11, 13, 12, 15, 17, 18, 19, 21, 22, 23), LAPS-PS (items 1, 2, 4, 5, 6, 7, 9), and LAPS-AR (items 3, 8, 14, 16, 20). The results were given as the sum of score. The handlers answered the questionnaire in the early stages of recruitment (appendix 1, 2).

Table 2. Descriptive demographics of the population of animal–assisted intervention participant dogs

	No.	Breed	Sex	Age	Weight	AAI experiences	Duration with handler	Living environment	animals living together
SAAA	S1	Maltese	female intact	5 yrs.	4 kg	3 months	3 yrs.	pet café	5 dogs
	S2	Shih Tzu	female neutered	3 yrs.	5 kg	6 months	3 yrs.	live with handler	–
	S3	Shetland Sheepdog	female intact	1 yrs.	7 kg	3 months	1 yrs.	live with handler	–
	S4	Shetland Sheepdog	female intact	3 yrs.	11 kg	6 months	2 yrs.	veterinary clinic	2 dogs
AKCAAT	A1	Mongrel	male neutered	9 yrs.	13 kg	19 months	9 yrs.	live with handler	2 cats
	A2	Chihuahua	male neutered	2 yrs.	6.3 kg	8 months	1 yrs.	live with handler	1 dog
	A3	Toy poodle	male neutered	3 yrs.	4.8 kg	24 months	3 yrs.	live with handler	1 dog
	A4	Toy poodle	male neutered	6 yrs.	7 kg	20 months	6 yrs.	live with handler	1 dog 2 ferret

Table 3. AAS scores of the participated handlers

Item	Description	S1	S2	S3	S4	A1	A2	A3	A4	Mean
#1	It is morally wrong to hunt wild animals just for sport	5	5	5	5	5	4	1	5	4.38
#2	I do not think that there is anything wrong with using animals in medical research	3	3	3	2	3	3	2	2	2.63
#3	I think it is perfectly acceptable for cattle and hogs to be raised for human consumption	3	5	4	2	4	3	3	2	3.25
#4	Basically, humans have the right to use animals as we see fit	2	3	3	2	1	2	3	1	2.13
#5	The slaughter of whales and dolphins should be immediately stopped even if it means some people will be put out of work.	3	4	2	4	4	3	4	5	3.63
#6	I sometimes get upset when I see wild animals in cages at zoos	3	3	3	3	5	4	4	4	3.63
#7	Breeding animals for their skins is a legitimate use of animals	1	2	4	3	1	1	1	1	1.75
#8	Some aspects of biology can only be learned through dissecting preserved animals such as cats	2	2	2	2	2	2	3	2	2.13
#9	It is unethical to breed purebred dogs for pets when millions of dogs are killed in animal shelters each year	3	2	4	2	5	3	3	3	3.13
#10	The use of animals such as rabbits for testing the safety of cosmetics and household products is unnecessary and should be stopped	3	3	3	5	5	3	4	3	3.63
Total		36	32	38	31	38	31	33	37	34.5

* Item #2, #3, #4, #7, #8: reversed calculated

** AAS: Animal Attitude Scale

Table 4. LAPS scores of the participated handlers

Item	Description	S1	S2	S3	S4	A1	A2	A3	A4	Mean
GA: General Attachment										
#1	My pet means more to me than any of my friends	2	2	3	2	3	2	3	3	2.5
#2	Quite often I confide in my pet	2	2	2	2	2	1	2	3	2
#4	I believe my pet is my best friend	2	3	2	2	3	2	3	3	2.5
#5	Quite often, my feelings towards people are affected by how they react to my pet	2	2	2	2	3	2	3	2	2.25
#6	I love my pet because he/she is more loyal to me than most of the people in my life	3	3	2	3	2	1	3	2	2.38
#7	I enjoy showing other people pictures of my pet	2	2	3	2	3	2	3	2	2.38
#9	I love my pet because it never judges me	1	3	2	2	2	2	3	3	2.25
#14	Pets deserve as much respect as humans do	2	3	2	2	3	2	3	3	2.5
GA average		2	2.5	2.3	2.1	2.6	1.8	2.9	2.6	2.34
PS: Person Substitution										
#10	My pet knows when I'm feeling bad	2	2	2	2	2	2	3	3	2.25
#11	I often talk to other people about my pet	2	2	4	2	3	2	3	3	2.63
#12	My pet understands me	2	2	2	2	2	2	2	3	2.13
#13	I believe that loving my pet helps me stay healthy	2	2	2	3	3	1	2	2	2.13
#15	My pet and I have a very close relationship	2	3	2	2	3	2	3	3	2.5
#17	I play with my pet quite often	2	3	2	2	3	2	2	3	2.38
#18	I consider my pet to be a great companion	2	3	2	2	3	2	3	3	2.5
#19	My pet makes me feel happy	2	3	2	2	3	2	3	3	2.5
#21	I am not very attached to my pet	2	1	1	2	1	0	0	0	0.88
#22	Owning a pet adds to my happiness	2	3	3	2	3	2	3	3	2.63
#23	I consider my pet to be a friend	2	3	3	2	3	2	3	3	2.63

PS average		2	2.5	2.3	2.1	2.6	1.7	2.5	2.6	2.28
AR: Animal Rights										
#3	I believe that pets should have the same rights and privileges as family members	2	3	2	2	3	2	3	3	2.5
#8	I think my pet is just a pet	3	2	2	2	2	1	0	0	1.5
#16	I would do almost anything to take care of my pet	2	2	3	2	3	2	3	3	2.5
#20	I feel that my pet is a part of my family	2	3	2	2	3	2	3	3	2.5
AR average		2.3	2.5	2.3	2	2.8	1.8	2.3	2.3	2.25
Total		47	57	52	48	64	44	65	62	54.9

* Item #8, #21: reversed calculated

** LAPS: Lexington Attachment to the Pets Scale; GA: General Attachment; PS: Person Substitution; AR: Animal Rights

2.2. AAI design

2.2.1. SAAA

The SAAA is a 4 week-program. Each session of a week took 50 minutes. During the first week's session, the handler introduced the rules and suggestions about the activity to participants before letting the participants meet the dog. The following sessions were 'learning understanding the emotional state of dogs', 'etiquette for dog walking, practicing dog walking', and 'participants reading to dogs'. During all AAI, the participants could interact with the dog under the supervision of the handler, such as hugging, touching, calling, and giving treats (Table 5). For welfare reasons, the dogs were able engage in resting activities such as lying down, drinking water, and going into a kennel without being restrained by their handlers during the AAI.

The SAAA was performed in regional children care facilities after school in Seoul. There was no separate rest area for the dogs. The size of the room where AAI was performed in two facilities was about 2.5 m × 6.2 m, and 4 m × 9 m. Each AAI started at 16:00 or 14:30. Parental and facility permission was obtained using oral informed consent. During each AAI, a supervising manager who was responsible for general safety was present. Each activity had two pairs of handler-dogs, one assistant, and six participants in room. There was no change in AAI participants' composition during the

study.

2.2.2. AKCAAT

The AKCAAT consists of 5 sessions. The schedule of the session could be changed according to the plan of the target organization, therefore AAI was not conducted every week. Each session took 50 minutes.

After the handler introduced to participants the rules about the activity, the participants could meet the dog in first session. Contents of following sessions were 'etiquette for dog walking', 'practicing dog walking', 'clicker training', 'decorating frame with memories with AAI dog', and 'playing treasure-hunting game', 'giant board game with a mission to do with animals'. At the end of every AAI session, participants could pet AAI dogs with treats for 10 minutes. During all AAI, the participants could interact with the dog under the supervision of the handler, such as hugging, touching, calling, and giving treats. The dogs were able to rest such as lying down, drinking water, and going into a kennel without being restrained by their handlers for welfare issue.

The AAI was performed at a residential facility for severely handicapped persons operated by the social welfare corporation "Yewon" in Incheon. The AAI started at 13:00, and the handler and the dog had to arrive at 12:30. There was no separate rest area for the dogs, however, because the time for rest were 30 minutes before

each AAI sessions, the dogs could relax in the AAI room or soiling in out of the facility. The AAI sessions were conducted in succession, followed by Autistic adult (13:00), Adult with intellectual disability (14:30), and Youth with Intellectual Disabilities (16:00). The size of activity room was about $4.5\text{ m} \times 7\text{ m}$. The average age of the participants was 30.7(Autistic adult), 31.7 (Adult with intellectual disability), 15.8 (Youth with Intellectual Disabilities) years, and there were six participants in each AAI class (Table 6). Parental and facility permission was obtained using oral informed consent. During AAI sessions, a supervising manager in facility who was responsible for general safety was present. Each activity had four pairs of handler–dogs, two assistants, and six participants. There was no change in AAI participants' composition during the study.

Table 5. Activities in the SAAA

Session	Activity details	Activity related with participant in dogs
1	Introducing the rules and suggestions about the activity to participants before letting the participants meet the dog	Learn to approach and touch
		Feeding and stroking
2	Learning how to express emotions in activity dog to understand the emotional state of activity dog	No direct activity, but exposed to the sound of folding the balloon
3	Improving self-esteem through obedience training and self-control through walking	Eye-gazing and Calling
		Sit command, Down command
		Take a lead and walk
4	Children reading to dogs	Children read the book to the dog
		Allow free contact with dog

Table 6. Participants of the AKCAAT

Group	description	Participant NO.	Gender	Age
Autistic adult	most of them consist of first grade of intellectual disability with autism symptoms	#1	female	50
		#2	female	24
		#3	female	27
		#4	female	30
		#5	male	24
		#6	male	29
Adult with intellectual disability	consists of adults who have received first and second grade intellectual disabilities	#7	female	34
		#8	female	37
		#9	female	22
		#10	male	24
		#11	male	23
		#12	male	47
Youth with Intellectual Disabilities	adolescents enrolled in school receiving first and second grade intellectual disabilities	#13	male	20
		#14	male	15
		#15	male	18
		#16	male	14
		#17	male	14
		#18	male	14

2.3. Salivary sample collection

Salivary samples were collected in four dogs (S1, S2, S3, S4) during 5 weeks from October 18th to November 15th, 2016 in the SAAA and four dogs (A1, A2, A3, A4) from April 21th to June 16th, 2017 in the AKCAAT. In case of the AKCAAT, there were baseline setting for identifying the influence of environmental and participate element on animal stress. In baseline setting dogs had relaxed only with handlers in their own houses, where dogs were not allowed to play roughly and take foods.

In every AAI pre-session (5 minutes before) and post-session (5 minutes after), a trained handler collected saliva from the partner AAI dog by using saliva-absorbing swabs (SalivaBio Children's Swabs, Salimetrics, State College, PA, USA) and saliva collection tubes (Salimetrics, StateCollege, PA, USA; Fig 1,2,3). Handlers sat next to the participating dogs and placed swabs under their tongue or in the side cheek pouch of the dog. The dogs were restrained for 90 to 120 seconds while chewing the swabs to allow production of saliva (Dreschel and Granger 2009). Treats were not used to prevent contamination of samples and to increase confidence in the results (Dreschel and Granger 2009). After confirming that the swab was sufficiently wet with the saliva, it was placed in a cooler containing dry ice before taking it to the laboratory. The saliva samples were frozen at -20° C until all sampling phases were completed and then analysed.

Fig. 1. Seoul Animal Assisted Activity Sampling Schedule

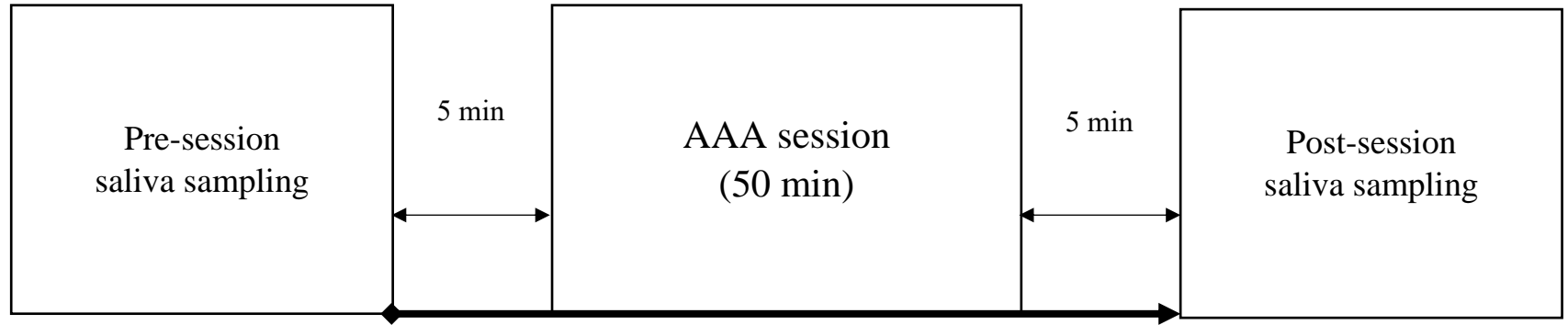


Fig. 2. Association of Korean Companion Animal Assisted Therapy Sampling Schedule

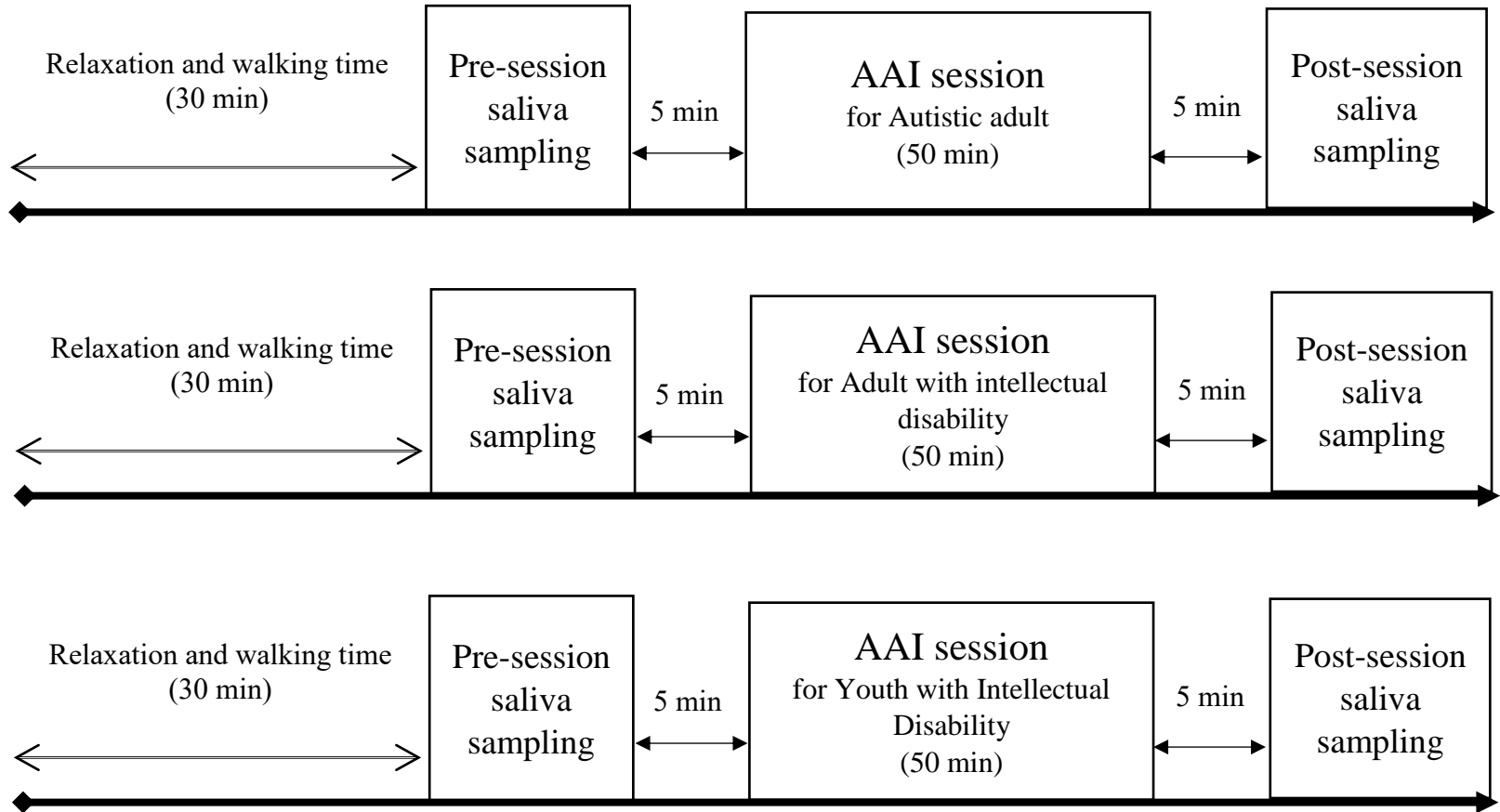
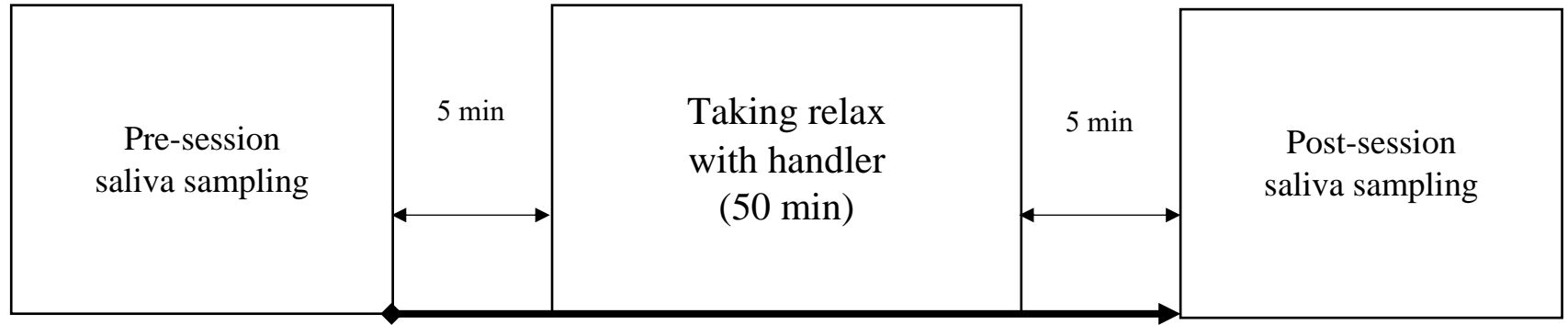


Fig. 3. Association of Korean Companion Animal Assisted Therapy Sampling Schedule in Baseline setting



2.4. Sample analysis

All samples were centrifuged at 3000 g for 15 min and cortisol levels were measured using a high-sensitivity enzyme immunoassay kit (Salimetrics SalivaryCortisol Immunoassay kit, Salimetrics, PA, USA). Following manufacturer's protocol, all cortisol standards, controls and saliva samples were assayed in duplicate in the plate by immunoassay. Cortisol standards, controls were included in the kit. If the sample volume fell below the limit needed to run duplicates, the sample was dismissed from the analysis. After reading the optical density (OD) of the plate wells at 450 nm on a plate reader, the average OD for all duplicate wells were calculated to the percent bound (B/Bo) by dividing the OD of each well (B) by the average OD for the zero (Bo). By interpolation on the cortisol standards value of B/Bo, the concentrations of the controls and saliva samples were calculated in using a 4-parameter nonlinear regression curve fit. Both average intra- and inter-assay coefficients of variance were less than 10%.

2.5. Statistical analysis

The level of cortisol measured in units of nmol/L at pre-session and post-session of the AAI were compared and tabled by dogs and dates. I used nonparametric statistics because the data were not normally distributed and the number of dog specimens was relatively small. Using the SPSS 24 for Windows (SPSS, Inc., Chicago, USA), Statistical analyses included Friedman two-way ANOVA and

Wilcoxon signed rank tests for the level of cortisol. To assess associations between the change of salivary cortisol levels and breeds, room, living condition, target population and session, Kruskal–Wallis tests were used. Scatterplots and Spearman correlation coefficients were used to assess correlation with change of salivary cortisol levels and weight, age, duration of ownership, AAI experience time, AAS and LAPS (total score of LAPS, LAPS–PS, LAPS–AR, LAPS–GA). For the AKCAAT, The Wilcoxon rank test was used to determine whether cortisol changes in the baseline and experimental setting were significantly different. Statistical significance was set at $p < 0.05$ in this study.

3. Results

3.1. SAAA programs

A total of 32 samples were collected from the 4 dogs (S1, S2, S3, S4). However, two samples were excluded from the analysis due to a lack of sufficient saliva. Enough saliva was obtained for 30 cortisol measurements. The average (median) of total cortisol levels was 6.984 (6.519) and 8.683 (7.163) nmol/L in pre-sessions and post-sessions of AAI activities, respectively (Fig. 4). In the comparison of pre-session and post-session cortisol levels, the p value in the Friedman two-way ANOVA was 0.637, and the p value in the Wilcoxon signed rank test was 0.679, indicating that there was no significant change in cortisol values between the pre-session and post-session. The changes in cortisol according to room ($p = 0.495$),

origins ($p = 0.89$), breed ($p = 0.79$) and session ($p = 0.204$) were not statistically significant. Differences in salivary cortisol level between sessions were not correlated with weight ($p = 0.547$), age ($p = 0.689$), AAI experiences ($p = 1$), duration of ownership ($p = 0.527$), AAS ($p = 0.954$), LAPS ($p = 0.954$), LAPS-PS ($p = 0.954$), LAPS-AR ($p = 0.954$), LAPS-GA ($p = 0.613$).

There were noteworthy increases of cortisol levels after session 3 in the dogs S2, S3, S4, although they are not statistically significant (Fig. 5). However, no accidents or unusual events were reported from handlers and facility managers on the date of session 3.

3.2. AKCAAT

A total of 136 samples were collected from the 4 dogs (A1, A2, A3, A4). However, there is a lack of sufficient saliva quantity in the dog A2 samples, 34 sample in that dogs were excluded from the analysis. Enough saliva was obtained for 102 cortisol measurements in 3 dogs (A1, A3, A4). The average (median) of total cortisol levels was 4.728 (3.736) and 4.625 (3.649) nmol/L in pre-sessions and post-sessions of AAI activities, and the average (median) of total cortisol levels was 2.351 (2.382) and 2.166 (2.140) nmol/L in pre-sessions and post-sessions of baseline respectively. In the comparison of pre-session and post-session cortisol levels, the p value in the Friedman two-way ANOVA was 0.529, and the p value in the Wilcoxon signed rank test was 0.341, indicating that there was no significant change in cortisol values between the pre-session and

post-session (Fig. 6).

There was no statistically significant difference in cortisol changes between the baseline and the AKCAAT setting ($p = 0.523$).

The changes in cortisol according to breed ($p = 0.94$), participants ($p = 0.887$), session ($p = 0.09$) were not statistically significant. Differences in salivary cortisol levels between session were not correlated with duration of ownership ($p = 0.051$), weight ($p = 0.051$), age ($p = 0.051$), AAI experiences ($p = 0.051$), AAI in month ($p = 0.051$), AAS ($p = 0.051$), LAPS ($p = 0.069$), LAPS-GA ($p = 0.333$). However, LAPS-PS ($p = 0.029$, Spearman's correlation coefficient = -0.227) and LAPS-AR ($p = 0.029$, Spearman's correlation coefficient = 0.227) has correlation with salivary cortisol in negatively and positively.

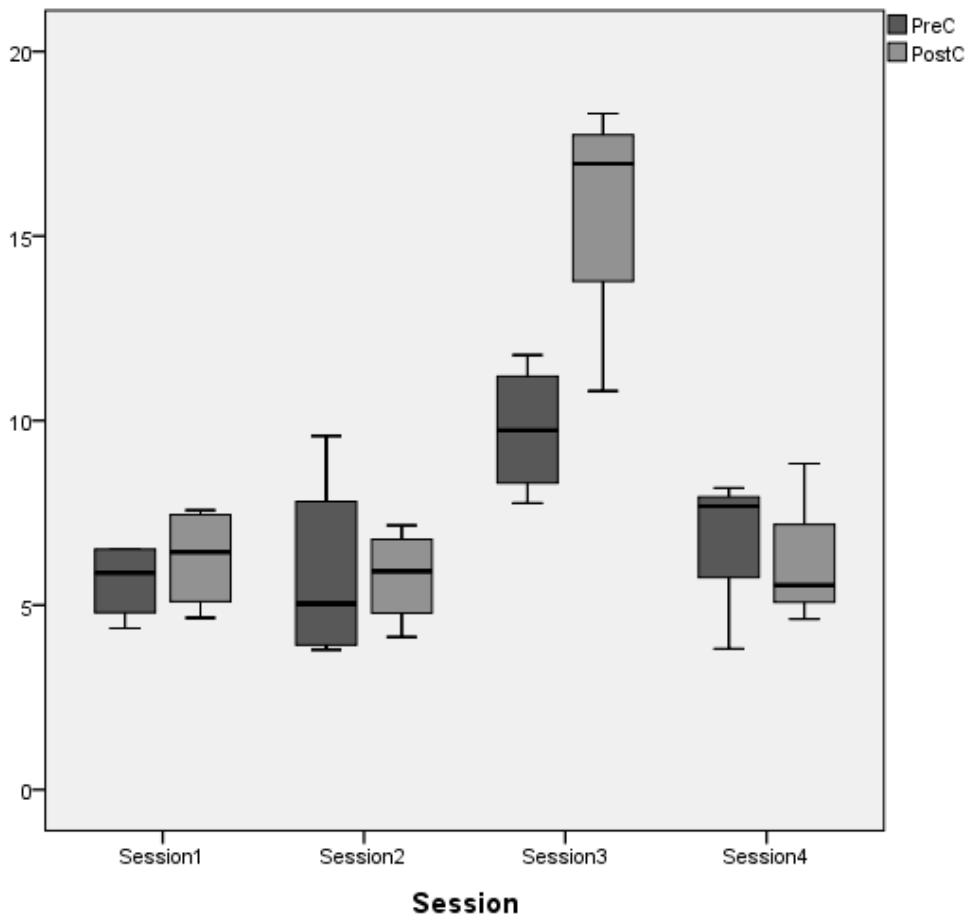


Fig. 4. Salivary cortisol levels of dogs in the SAAA by session (N=4)

The changes in cortisol according to session ($p = 0.204$) were not statistically significant.

· Unit: nmol/L; PreC: Pre-session; PostC: Post-session

· Session 1: introducing the rules and suggestions about the activity to participants before letting the participants meet the dog; Session 2: learning how to express emotions in activity dog to understand the emotional state of activity dog; Session 3: improving self-esteem through obedience training and self-control through walking; Session 4: children reading to dogs

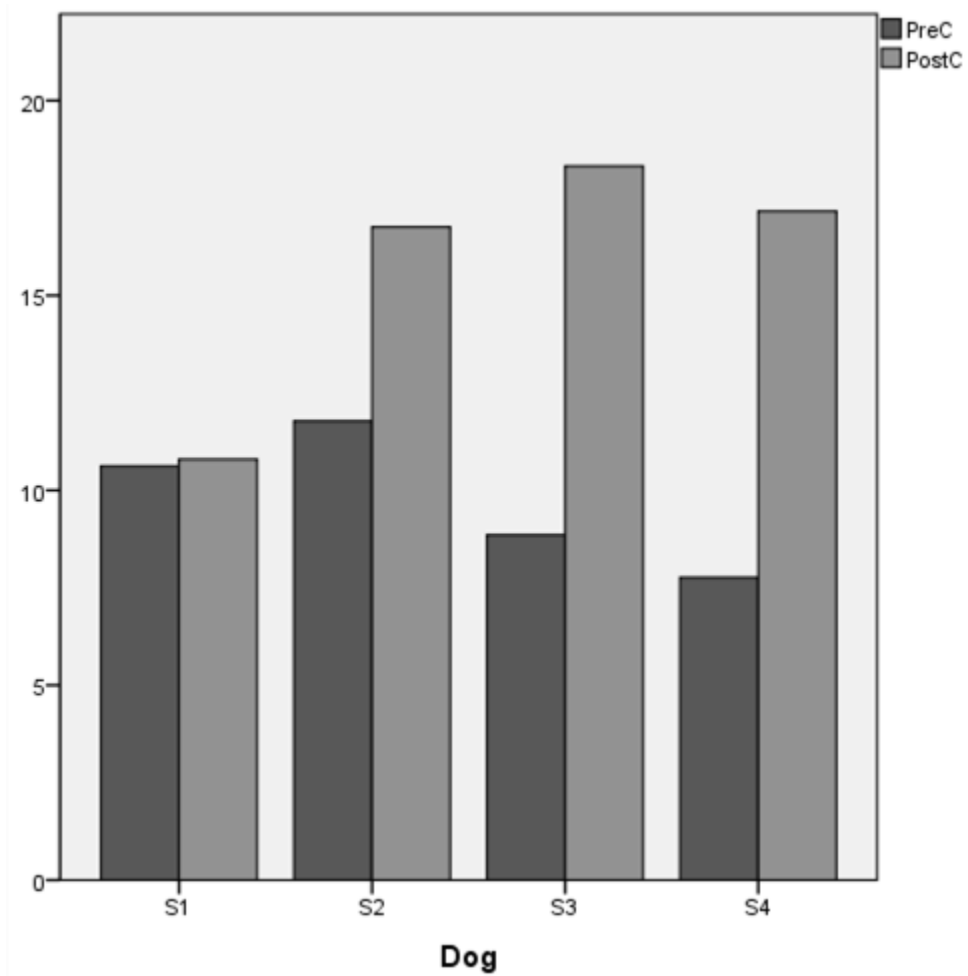


Fig. 5. Salivary cortisol levels of dogs in session 3 of the SAAA

There were noteworthy increases of cortisol levels after session 3 in dogs S2, S3, S4.

· Unit: nmol/L; PreC: Pre-session; PostC: Post-session

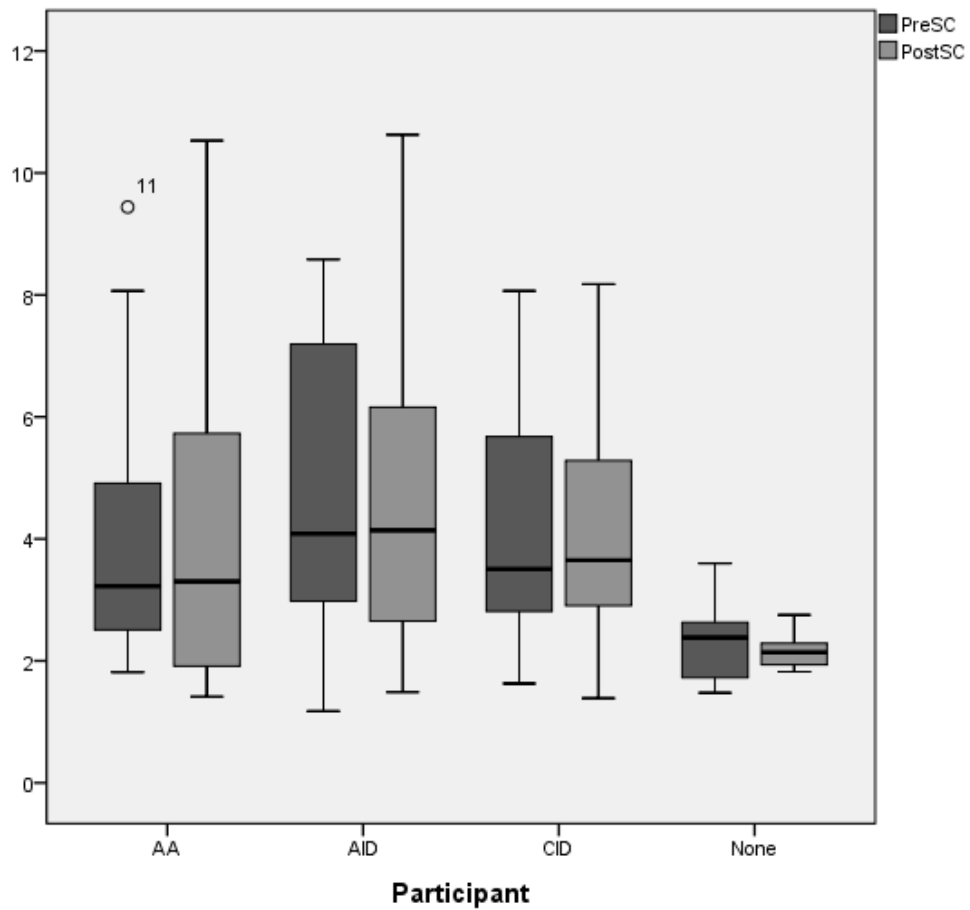


Fig. 6. Salivary cortisol levels of dogs in the AKCAAT by participant

The changes in cortisol according to participants ($p = 0.887$) were not statistically significant

· Unit: nmol/L; PreC: Pre-session; PostC: Post-session;

·AA: Autism Adult, Most of them consist of first grade of intellectual disability with autism symptoms; AID: Adult with intellectual disable, Consists of adults who have received first and second grade intellectual disabilities; CID: Children with intellectual disable, Adolescents enrolled in school receiving first and second grade intellectual disabilities; None: baseline setting

4. Discussion

This study compared the AAI dogs stress levels in different kinds of activities and examined the stress level of AAI dogs according to the characteristics of the participants. In SAAA for children by volunteers with pets, I examined cortisol levels of the four AAI dogs to compare the stress level in different kinds of activities contents. In AKCAAT by professional handler and dogs, I compare the stress level of AAI dogs according to the characteristics of the participants, the four dogs which have activities with three groups of intellectual disabilities (autistic adult, adult with intellectual disability, and youth with intellectual disabilities) were examined. Although there were no significant changes in the salivary cortisol as physiological indicator, the activities demanding strong human–animal relationships increased level of stress in AAI dogs, especially young and inexperienced dogs.

Overall, the absence of elevated cortisol levels is likely due to the fact that these dogs were selected dogs for AAI activity. Rosado's study showed that aggressive dogs had a more significant association with plasma cortisol levels than non–aggressive dogs (Rosado *et al.* 2010).

A high cortisol levels in dogs indicates that the dog is in a state of high stress, and measuring cortisol is critical in identifying dog welfare states (Coppola *et al.* 2006; Bergamasco *et al.* 2010). A rise of cortisol was proved to be associated with a stressful state caused

by fear (Hydbring–Sandberg *et al.* 2004; Dreschel and Granger 2005). In this study, the higher cortisol level after session 3 can be explained by the activities that may have been linked to fear or anxiety. In session 3, the main activities were practicing obedience training and walking with the dog. Although the frequency of direct contact with participants in this session did not increase, there were activities involving command execution and walking that did not exist in former sessions. It can be assumed that these activities may have made human–animal interactions during session 3 stressful, as opposed to quiet play, petting, and affiliate behaviours that reduce cortisol levels in dogs (Horváth *et al.* 2008; Shiverdecker *et al.* 2013). Coercive interactions with humans that include pushing, yelling, and punitive actions or threats elevate cortisol in dogs (Jones and Josephs 2006; Horváth *et al.* 2007).

Since dogs usually belong to one or a particular group of people and have been changed during the process of domestication to help people in work such as hunting and guarding, they have a habit of warning against strangers or intruders (Butler 2004). Therefore, AAI, which involves interacting with strangers in a strange place and with a variety of interactions, can cause stress to dogs, which can hinder animal welfare and even harm their health (Hatch 2007; Houpt *et al.* 2007; Serpell *et al.* 2010). Researchers have pointed out that these activities can cause fatigue or burnout in working dogs (Iannuzzi and Rowan 1991). In addition, performing interactions and obedience commands beyond simple petting, and restricting movement during

AAI, may have contributed to the increase in cortisol levels in session 3 (Glenk *et al.* 2013).

During the AAI sessions, the participating dogs could move without restriction and escape the setting of the activity by avoiding contact with human beings or entering a kennel. However, since there were specific activity goals using dogs in session 3, the AAI dogs could not avoid contact with humans. The presence or absence of this freedom of choice to seek human contact in dogs can affect differences in cortisol levels (Glenk *et al.* 2014). The freedom of animals, which allows them to voluntarily decide whether or not to interact, appears to be another important factor in animal welfare. This needs to be treated as an important variable in subsequent studies.

In the SAAA, the dog S1 did not show an increase in cortisol at session 3. Unlike other dogs, the S1 has the characteristics of living in an animal cafe and being older than other dogs. From these characteristics, it can be inferred that aged dogs are less affected by stress on the interaction that requires a strong human–animal bond to the stranger. The maturity that comes from age and the many socialization experiences can be thought of as giving resistance to these parts. The tendency based on this age also was shown in the AKCAAT dogs. The difference in cortisol changes between the groups above 5 years and under 5years was by the Kruskal–Wallis test ($P = 0.034$). Glenk suggested that the suitability of an animal in AAI may vary with its lifespan (Glenk *et al.* 2017). Animal handlers

responded that aged dogs have a milder temperament and therefore may be more suitable for AAI (Hatch 2007), and in actual studies, older dogs showed less stress response and behavior (King *et al.* 2011).

An animal cafe is a commercial exhibition facility for profit-making, which raises other animals such as dogs and cats in a cafe space and allows visitors to pet and treat animals in cafes and sell treats and drinks to them. Thus, unlike dogs living at home, the dog S1 became a desensitization and habituation to the human-animal interaction with a stranger. Because of this background, the dog S1 appears to show no increase in cortisol in the AAI process, which requires high human-animal bonds as in session 3. On the other side, dogs that have not been adapted to an interaction with a stranger may be more likely to be stressed when doing activities that require intense human-animal bonds. However, there is no standardized training process for AAI in Korea, therefore there is no opportunity for dogs to adapt and prepare for the new environment. Thus, to reduce the stress caused by the difference in experience and age, a system of adaptation training to the social environment of AAI will be needed for newly participating dogs in AAI.

Although dogs did not show an increase in cortisol suggesting the occurrence of stress in the AAI process for children covered in this study, the welfare of dogs participating in AAA should be monitored and evaluated on an ongoing basis. A single AAI session does not cause an acute stress response, it does not know how much stress

can be induced by the duration or frequency of an AAI session, and it may interfere with the homeostatic mechanism and chronic stress over time (Karatsoreos and McEwen, 2011).

Salivary cortisol reactions and a very low posture to the ground tend to be associated with more severe acute stress states in dogs (Beerda *et al.* 1998). However, signs such as restlessness, moderate lowering, and elevated levels of oral behaviour, which are increased when experiencing mild stress in a social setting are not related to cortisol responses. Cortisol may be less sensitive to stress from social contexts such as human–animal relationships and restraints when used as a stand-alone indicator. Also, care should be taken when interpreting salivary cortisol results with biomarkers of welfare assessments, because increased levels of cortisol reflect both positive excitement and negative stress (Zenithson *et al.* 2014). Additional assessment of behaviour and the heart rate in addition to the cortisol level is needed to determine the negative states that affect animal welfare.

It has been suggested that the setting of interventions and the interaction of human and animal characteristics with these variables influence the outcome of AAI (Wilson and Barker 2003). These results can be thought of not only on humans but also on animals. In the case of subjects with dementia or autism, which is the subject of AAI, aggressive behavior can be seen in interaction with animals during AAI (Bridges–Parlet *et al.* 1994; Farmer and Aman 2011).

Therefore, it is also important to measure the effects of these uncontrollable and non-actionable behaviors on AAI outcomes.

According to Marinelli's researchers, stress-related reactions were more frequent in sessions with children under 12 years of age. However, this study did not show such a tendency in this study for children. It is important for future studies to assess stress by setting a control group and varying age groups to put participants' age as a variable for stress in AAI dog. Marinelli and colleagues have also pointed out that elevated temperature and space shortages are inadequate environmental conditions for dog welfare (Marinelli *et al.* 2009). Also, Hydbring found that walking on a different footing than usual could cause physiological arousal even in dogs that had not previously been afraid. (Hydbring *et al.* 2004). In this study, however, there was no change in the response of cortisol to space conditions. Future research will be needed to establish guidelines for the various stressors in AAI by setting up more various conditions.

ZaiTiir questioned the moral legitimacy of AAI in associating animals in dealing with the subject (ZaiTiir, 2006). ZaiTiir proposed six ethical violations, including the limitations of freedom, life determination, training, social disconnect, injury, and instrumentation. However, animal participation in AAI can be ethically justified if these concerns can be managed and animals can benefit from human interactions (Glenk, 2017).

It has been reported that non-professional samplers such as dog handlers can easily perform saliva cortisol sampling (Dreschel and

Granger 2009). However, as shown by King (2011) and Glenk (2013), dog handlers trained in saliva sampling can also fail to sample or have difficulties. In these cases, even if the sampling is successful, the amount of saliva collected may not be sufficient to cause an antibody reaction. To avoid unmeasurable samples and to obtain sufficient salivary sample quantities for analysis, it is helpful to collect samples from people who have received training and can be evaluated through a pilot study.

There is no statistically significant difference in cortisol level between baseline and AAI setting, but there is a large difference in value in pre-session. It is estimated due to unfamiliar external environment and transportation. AKCAAT handler and dogs moved to facility by Vans. Dogs can be stressed when transported by car (Kuhn, 1990). Given the fact that dogs can be stressed when moving for AAI purposes, further studies are needed to measure changes in cortisol levels as a function of transportation methods and travel time factors.

Measuring stress during actual AAI activities, rather than in a designed or controlled environment, may result in an approximation of the real-world target. However, in a future study, the influence of the environment and conditions need to be detailed and clarified, such as the characteristics of the participants, the experience of the AAI dogs, place familiarity, moving distance, activity content, and resting time.

Regarding LAPS–PS and LAPS–AR, although the results of handler's attachment to dogs were found to affect the change in dog stress, they showed different correlation coefficients. The reason of this opposite orientation seems to be the limit for a small number of handler–dog groups. Therefore, the search for the direction of this influence needs to be done more closely in subsequent studies.

Animal–assisted intervention may be beneficial to humans by improving their welfare through interactions with therapeutic dogs (Barker *et al.* 2003). In order for AAI to have a positive impact, it is necessary to keep the animals participating in the intervention physically and behaviorally healthy (Glenk *et al.* 2014), and the dog handler has a moral obligation to guarantee animal welfare (Rooney *et al.* 2009). The American Association for Veterinary Medicine, Association for Animal Interaction, International Society for Animal–Assisted Therapy, European Society for Animal Assisted Therapy, and European Standard Therapy Dog Training suggest basic guidelines that provide a list of primary considerations for maintaining animal welfare during the AAI period by closely monitoring the signs. SAAA also provides tables about the signs of stress in educational materials manuals to handlers. However, there is no standardized manual to deal with participating animals that have been agreed upon in the AAI field. It is necessary for each handler participating in the activity or managing the program to be properly trained in recognizing behaviors that can be seen in a stressful situation, and in preventing and managing them, while the dog is

aware of the usual behavior (Mariti *et al.* 2012).

Individual welfare states are related to the ability to deal with the stress imposed by the environment (Wechsler 1995). In AAI, these environmental factors include space, target population, handler, and activity context. However, there is no single validation model available to determine the effect these factors have on a dog (Palestrini *et al.* 2017). The reason why there is no standard model in academic studies is that structural elements in each AAI vary by situation and activity environments. There are differences between AAI programs regarding dog training procedures, AAI work schedule, facility arrival and start times, quality assessment, and quality assurance. The number of studies on animals participating in AAI is insufficient to overcome these variations. Therefore, there is a lack of academic data to provide a scientific basis for making and publishing standards on animal welfare in AAI (Beck and Katcher 2003). As a result, it is important to increase knowledge of measurable variables that reflect aspects of animal welfare and to provide evidence for which criteria should be met during AAI. The results of this study can contribute to enriching data about environmental and demographic variables in AAI. Measuring the stress of participating dogs and the influence of AAI variables in the field is meaningful for analysing interactions between dogs and environmental factors.

In the case of AAI involving dogs, consideration should be given to

the fact that biased populations may be the subject of research through the screening process for preparing AAI. However, since dogs participating in the AAI can receive certificates and participate in activities only when they meet certain criteria through careful behavior assessment and training (Serpell *et al.* 2010), the sample collected in this study clearly reflects the target population.

The stresses received during the sampling process do not affect short-term measurements, as they take 15 minutes to be applied to actual plasma and saliva (Vincent and Michell, 1992; Zenithon, 2014). However, since the purpose of the study is to see the trend of cortisol changes, it is important to avoid giving too much stress during the sampling process to obtain accurate results. Therefore, it is important to keep in mind that handler did not perform saliva sampling when an activity dog rejected so that the result can be biased. This bias may lead to the exclusion of more sensitive or contact-stressed individuals, as opposed to measuring the actual AAI population targeted, because of modeling studies of subjects who have undergone trained or behavioral assessment as discussed above. In future research, habituation of the sampling process at the pre-study phase or the AAI training phase will minimize dog responses by the impact of the sampling.

The pattern of cortisol response is complex and the magnitude, duration, and rate of change vary individually. Salivary cortisol levels reflect plasma cortisol levels with a delay of 20 to 30 minutes

(Wenger B *et al.* 2010). Cortisol production peaks at the first cycle and falls to the lowest level at night. Independently of the rhythm of this cycle, the level rises in response to stress. Therefore, sampling in the afternoon, avoiding the morning hours of cortisol, may show less error. Based on the reason, cortisol measurement for AAI involving dogs did on an afternoon in this experiment.

5. Conclusion and animal welfare implications

In this study the cortisol level of dogs participating a 50-minute AAI session for children and a 50-minute AAI session for people with Autism and the intellectual disabled in Korea did not significantly increase. However, it was noted that some activities that need stronger human-animal relationships can be stressful to the AAI dogs. Monitoring and analysing both physiological and behavioural parameters related to animal welfare are essential to provide consistently high quality in AAI (Palestrini *et al.* 2017).

5.1. Acknowledgments

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5.2. Conflict of interest

There were no conflicts, concerns, or objections during the study.

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Appendix 1: Animal Attitude Scale Questionnaire

동물 태도 측정 (Animal Attitude Scale) 설문지

문항	절대 동의하 지 않음	동의하 지 않음	동의하 는 편임	동의함	전적으 로 동의함
1. 단지 스포츠로 야생동물을 사냥하는 것은 윤리적으로 옳지 못하다.	1	2	3	4	5
2. 나는 의학 연구를 위해 동물을 사용하는 것에는 어떠한 문제도 있다고 생각하지 않는다. **	1	2	3	4	5
3. 나는 식용을 위해 소와 돼지를 키우는 것은 완벽하게 받아들일 수 있다. **	1	2	3	4	5
4. 기본적으로, 인간에게는 원하는 대로 동물을 이용할 권리가 있다. **	1	2	3	4	5
5. 비록 일부 사람들이 직업을 잃게 되더라도, 고래 포획은 즉각적으로 중단되어야만 한다.	1	2	3	4	5
6. 동물원 우리 속의 야생동물을 볼 때 나는 가끔 화가 난다	1	2	3	4	5
7. 가축을 얻기 위해 동물을 기르는 것은 정당하다. **	1	2	3	4	5
8. 생물학의 특정 분야의 경우, 오직 고양이 같은 동물의 해부를 통해서만 그 지식을 얻을 수 있다. **	1	2	3	4	5
9. 매년 동물 보호소에서 수백만 마리의 개가 안락사 당하는 와중에 품종견을 반려동물로 기르는 것은 윤리적으로 옳지 못하다.	1	2	3	4	5
10. 화장품이나 가정용품 등의 안전성을 검사하는데 토끼 등의 동물을 사용하는 것은 불필요하며, 중단되어야 한다.	1	2	3	4	5

Appendix 2: Lexington Attachment to Pets Scale Questionnaire

반려동물 관계도(Lexington Attachment to Pets Scale) 설문지

문항	절대 동의하지 않음	동의하지 않음	동의함	전적으로 동의함
1. 내 반려동물은 어떤 친구들보다 나에게 의미가 있다.	0	1	2	3
2. 꽤 자주 내 반려동물에게 비밀을 털어 놓는다.	0	1	2	3
3. 나는 반려동물이 가족구성원들과 마찬가지로의 권리를 가져야 한다고 믿는다.	0	1	2	3
4. 내 반려동물은 내 가장 친한 친구이다.	0	1	2	3
5. 꽤 자주, 사람들이 내 반려동물에게 대하는 방식은 그 사람들을 향한 내 감정에 영향을 미친다.	0	1	2	3
6. 나는 내 반려동물이 내 인생의 어떤 사람보다 나에게 충실하기 때문에 내 반려동물을 사랑한다.	0	1	2	3
7. 나는 다른 이들에게 내 반려동물 사진을 보여주기를 즐긴다.	0	1	2	3
8. 내 반려동물은 그저 반려동물일 뿐이다.	0	1	2	3
9. 나의 반려동물은 절대 나에 대해 판단하지 않기 때문에, 나는 내 반려동물을 사랑한다.	0	1	2	3
10. 내가 기분이 안 좋을 때 내 반려동물은 이를 알고 있다.	0	1	2	3
11. 나는 때때로 다른 사람들에게 내 반려동물에 대해 이야기한다.	0	1	2	3
12. 내 반려동물은 나를 이해한다.	0	1	2	3
13. 반려동물을 사랑하는 것은 내가 건강을 유지하는데 도움이 된다.	0	1	2	3
14. 반려동물은 인간이 존중받는 것만큼 존중받아야 한다.	0	1	2	3
15. 내 반려동물과 나는 굉장히 가까운 관계이다.	0	1	2	3
16. 나는 내 반려동물을 돌보기 위해 무엇이든 할 수 있다.	0	1	2	3
17. 나는 내 반려동물과 꽤 자주 같이 논다.	0	1	2	3
18. 나는 내 반려동물이 훌륭한 동반자라고 생각한다.	0	1	2	3
19. 내 반려동물은 날 행복하게 해준다.	0	1	2	3
20. 나는 내 반려동물이 내 가족의 구성원이라고 느낀다.	0	1	2	3
21. 난 내 반려동물과 매우 친밀하지는 않다.	0	1	2	3
22. 반려동물을 가지는 것은 내 행복을 더 해준다.	0	1	2	3
23. 나는 내 반려동물을 친구로 생각한다.	0	1	2	3

국문초록

비록 동물매개중재활동(AAI)에서 대상자인 인간에 대한 질 표준 및 위험 관리는 수 년간 조사되어 왔지만, AAI에 참여하는 동물의 복지에 관해서는 아주 적은 수의 연구만이 존재하고 있다. 동물과 관련된 AAI에 대한 동물 복지 기준을 설정하려면 실무 활동에서 동물의 스트레스 수준에 대한 연구가 필요하다. 이 연구에서는 여러 종류의 활동에서의 스트레스 수준을 비교하기 위하여, 반려 동물과 함께 참여한 자원봉사자가 진행하는 SAAA에서의 4마리의 AAI 개의 코티졸 수치를 조사하였다. 또한, 참가자의 특성에 따라 AAI 개의 스트레스 수준을 비교하기 위해 AKCAAT 활동에서 지적 장애 (자폐증 성인, 지적 장애가 있는 성인 및 지능 장애가 있는 청소년)의 세 그룹을 대상으로 활동하는 4마리의 개를 조사했다. 생리 지표인 타액 코티졸에서는 유의미한 스트레스 변화는 관찰되지 않았지만, 강한 인간-동물 관계를 요구하는 활동은 젊거나 경험이 없는 개에서 스트레스를 증가시키는 것으로 나타났다. 이 연구 결과는 한국의 AAI 동물의 스트레스 수준 모니터링과 동물 복지 지침 설정에 대한 통찰력을 제공 할 수 있다.

키워드

동물매개활동, 동물매개개입, 동물복지, 노동동물, 스트레스, 코티졸